Rapid communications

Analysis of the HepG2/erythrocyte glucose transporter locus in a family with Type 2 (non-insulin-dependent) diabetes and obesity

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Summary. A recent report has shown an association between a specific Xba1 restriction fragment of the human HepG2-Erythrocyte glucose transporter gene and Type 2 (non-insulin dependent) diabetes. To further examine the significance of this finding we have studied Type 2 diabetic pedigrees for linkage between the Xba1 alleles of this glucose transporter gene and diabetes. One large pedigree, in which the diabetic phenotype was associated with obesity and insulin resistance, was informative. In this family the disease did not co-segregate with the glucose transporter locus. Formal linkage analysis was performed assuming autosomal dominant inheri-

tance with age-dependent penetrance. At putative gene frequencies of 0.01 and 0.001 the logarithm of the odds for linkage versus non-linkage at a recombination fraction of 0.001 was -1.84 and -3.32 respectively (a value of <-2 indicates definite non-linkage). Genetic variations in the HepG2-Erythrocyte glucose transporter gene are unlikely to be responsible for the development of diabetes in this pedigree.

Key words: Type 2 (non-insulin-dependent) diabetes, genetics, linkage, glucose transporter.

Type 2 (non-insulin-dependent) diabetes is characterised by both defective insulin secretion and action with considerable controversy as to which feature might be primary. Resistance to the effects of insulin is consistently found in established diabetes but might result from, rather than be the cause of, hyperglycaemia. In-vivo and in-vitro studies in Type 2 diabetes have suggested that the defect producing insulin resistance is likely to be distal to the binding of insulin to its receptor [1]. It is possible that in some Type 2 (non-insulin-dependent) diabetic families, especially in certain racial groups such as Pima Indians [2], a defect of insulin action might be inherited. A family of glucose transporter proteins is being characterised and immunochemical studies in sub-cutaneous adipocytes of Type 2 diabetics subjects suggest a reduction in intracellular transporters [3]. The first of the glucose transporters for which the gene has been isolated and characterised is the human HepG2/erythrocyte glucose transporter (GLUT) [4]. Li et al. have recently reported

an association of an Xba1 restriction fragment length polymorphism (RFLP) of the GLUT gene with diabetes in three separate racial groups and have concluded that a diabetogenic locus may lie within or near the gene [5].

If the GLUT locus is a major diabetogenic locus then GLUT alleles should co-segregate with the disease in affected pedigrees. We have studied the inheritance of GLUT alleles in families in which Type 2 diabetes appears to be inherited as an autosomal dominant.

Subjects and methods

Family AX

Five large families were studied and only Family AX was informative for the RFLP. The proband (IV 4) was ascertained through a diabetic clinic and all relatives known to have diabetes were contacted and

Table 1. Clinical details of diabetic members of family AX. Most diabetic members of the family are obese

Subject	Sex	Diabetic status	Age at diagnosis	Duration of diabetes (years)	Body mass index	Presenting symptoms	Initial therapy	Time on initial therapy (years)	Current therapy	Complications
II 6	F	D	81	8	-	Thirst	Diet	8	Diet	Cataracts
II 9	F	D	68	15	-	-	Insulin	15	Insulin	-
III 2	F	D	31	44	43.4	Polyuria	Insulin	44	Insulin	Retinopathy, Neuropathy
III 5	M	D	44	10	-	Incidental	Diet	1	Diet	-
III 7	M	D	48	14	27.4	-	Diet	1	OHA	Neuropathy
III 8	M	D	70	3	25.4	Incidental	Diet	3	Diet	None
III 15	M	IGT	54	2	24.3	Incidental	-	-	-	None
III 16	M	D	53	2	21.0	Incidental	Diet	3	Diet	None
IV 2	F	D	36	3	37.9	Incidental	Diet	0.25	OHA	None
IV 4	F	D	51	5	47.2	Incidental	Diet	2	OHA	None
IV 5	F	D	44	9	41.2	Pruritus vulvae	Diet	4	OHA	Retinopathy
IV 6	F	D	31	2	-	In pregnancy	Diet	2	Diet	None

OHA - oral hypoglycaemic agents; IGT - impaired glucose tolerance

Fig. 1. Family tree of studied members of Family AX showing the inheritance of glucose transporter alleles. Affected subject III2 transmits the B allele to two affected daughters and the A allele to the other affected daughter. Subject III5, the affected brother of III2 also transmits an A allele to his affected daughter. Diabetes clearly does not segregate with either the A or B glucose transporter allele in this family. Symbols are as follows (Squares = males and circles = females). ■ Diabetic; ▨ Glucose intolerant; □ Normal glucose tolerance; □ Normal fasting plasma glucose; ∕ Dead; □ Not tested

Restriction fragment length polymorphism

A cDNA probe hGT2-1 representing an Eco R1 fragment spanning nucleotides -200 to 281 was used to detect an RFLP with alleles of 6.5 (A) and 6.3 (B) kb in genomic DNA digested with Xba1 (Boehringer Research Laboratories, Paisley, UK) and Southern blotted onto nylon filters (Hybond N, Amersham, UK).

Statistical analysis

Linkage analysis. The pedigree was initially inspected for any recombinants between diabetes and the glucose transporter locus, and was subsequently subjected to formal LOC (logarithm of the odds for linkage versus non-linkage) score analysis using the Linkage [7] package. Age-related penetrance was taken into account in the analysis as described [8]. Linkage analysis was performed with putative gene frequencies of 0.001, 0.01 and 0.05. As the hypothesis being tested is that genetic defects in the glucose transporter locus itself have a causative role in this family the LOD (logarithm of the odds for linkage versus non-linkage) scores are presented for a recombination fraction of 0.001 only. This represents a region of Chromosome 1 of approximately 100 kilobases around the GLUT gene.

asked to provide a blood sample. All other available family members were tested with either a fasting plasma glucose or a continuous infusion of glucose test [6]. The family was examined for paternity using DNA fingerprinting techniques using probes p33.15 and p33.6 [13]. Table 1 shows the clinical details of the affected members of family AX. The criteria for "affected" and "unaffected" have been reported in detail [6]. Five of eight affected family members for whom data are available were obese (Body Mass Index (BMI) > 27) with BMI > 30 in four. Insulin resistance is suggested by available data i.e. subject III2 required > 100 U of insulin per day to control diabetes; subject IV4 and IV5 had elevated fasting plasma insulin at 11.2 m and 20.3 mU/l respectively (normal range 2-7 mU/l) and two of the teenage offspring of IV4 (not included in linkage analysis) had high fasting plasma insulin (14.2 and 11.8 mU/l).

Results

Figure 1 shows the inheritance of the GLUT alleles in pedigree AX. Inspection of the pedigree shows that there are several recombinants between the GLUT locus and diabetes. Subject III2, who is both diabetic and markedly obese transmits the B allele to two of her similarly affected daughters but the A allele to the other affected daughter. Subject III5, the affected brother of III2, also transmits allele A to his affected daughter. Several affected individuals in the pedigree do not share a GLUT allele. At all assumed gene frequencies the LOD score was negative but statistical proof of non-linkage was obtained only at a gene frequency of 0.001 (LOD score -3.47) although at a gene frequency of 0.01 the data approached significance (LOD score -1.84). At a gene frequency 0.05 the LOD score was -0.84 .

Discussion

A genetic locus for susceptibility to Type 2 diabetes has not yet been characterised. Associations of specific alleles of candidate genes, such as the insulin and insulin receptor genes, with diabetes have been reported but linkage studies in pedigrees have given negative results [8]. Li et al. recently demonstrated an association between a 6.2 kb Xba1 RFLP of the HepG2/Erythrocyte glucose transporter (GLUT) gene and Type 2 diabetes [5]. Large numbers of subjects were studied and the results were consistent in three separate racial groups. They suggest that a major diabetogenic locus may lie in or near the GLUT locus on Chromosome 1. This hypothesis requires further testing by examination of diabetic pedigrees for linkage between diabetes and the GLUT locus. We have examined five large diabetic pedigrees, only one of which was informative for the Xba1 RFLP. In this family, which is characterised by considerable obesity, co-segregation was not found, although formal proof of non-linkage required the assumption of a low gene frequency for this form of diabetes. The GLUT locus has limited polymorphism and it is likely to be difficult to find large informative families. This family has already been examined for linkage with the insulin receptor locus and the presence of recombinants made a causative role for the insulin receptor gene unlikely although the LOD score did not reach statistical significance [8].

The credentials of the GLUT gene as a candidate gene in diabetes need to be taken into account. The HepG2/erythrocyte glucose transporter is one of a family of glucose transporter proteins and indeed does not appear to be the major transporter in insulin sensitive tissues [9]. It is expressed to a high level in placenta, and to a lesser extent in kidney, brain, gastro-intes-

tinal tract and adipose tissue. Fat cells probably contain two glucose transporters which are immunochemically distinct. The one which it shares with skeletal muscle primarily resides in an intra-cellular compartment and is translocated to the cell surface on exposure to insulin whereas the other may be constitutively expressed in the plasma membrane of resting fat cells [8]. The relationship between these immunologically and functionally distinct transporters and the GLUT gene is unclear. The transporters could be coded by separate genes, or could result from alternative mRNA splicing or post-translational modification. The gene under investigation in this report may not code for the glucose transporter in muscle, which is the major insulin sensitive tissue.

The use of linkage analysis to study a disease whose mode of inheritance is uncertain is controversial. Several workers have concluded that the susceptibility to Type 2 diabetes is likely to be inherited as an autosomal dominant [6]. In this family the disease segregates as a dominant trait and we consider that formal linkage analysis is appropriate.

We examined a family whose diabetes is characterised by obesity and insulin resistance. We have previously suggested that the primary defect in many Type 2 diabetic families resides in the B cell [10] but genetic heterogeneity is possible with some families having major defects of glucose uptake in peripheral tissues. Further families should be studied with this and other glucose transporter genes. If the insulin regulatable glucose transporter of muscle and fat proves to be coded by a separate gene then this should have high priority for linkage analysis studies in families whose phenotype suggests marked insulin resistance.

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Note added in proof

The cloning of the cDNA codint for an insulin-regulatable glucose transporter has recently been reported. The DNA sequence is different from that of the HepG2/erythrocyte transporter but its chromosomal localization is, as yet, unreported. [James DE, Strube M, Muechler M (1989) Molecular cloning and characterization of an insulinregulatable glucose transporter. *Nature* 333: 83-87]